PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

RECEIVED
01 MAR 2004
WIPO PCT

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
31661PC01		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB 03/00028	09.01.2003	09.01.2002
International Patent Classification (IPC) or both national classification and IPC		
C12N15/53		
Applicant		
AREXIS AB		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.08.2003	25. 02. 2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Hornig, H Telephone No. +31 70 340-2620

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/00028

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-19 and Sequence Listing 1/13-13/13	as originally filed

Claims, Numbers

1-7, 9, 12, 14	as originally filed
8, 10, 11, 13	received on 30.01.2004 with letter of 30.01.2004

Drawings, Sheets

1/1	as originally filed

Sequence listing part of the description, pages:

1-13, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10-14

because:

☒ the said international application, or the said claims Nos. 10-14 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

1.1 The amended claims 8, 10, 11 and 13 filed with the letter dated 30.01.04 and received on 30.01.04 are allowable according to Art. 34 (2)(b) PCT. The basis of the report issues on the claims as amended according to Rule 70.2 PCT.

Re Item III

Claims 10-14 relate to subject-matter **(..obtaining a ... sample..)** considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34 (4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: WO 01 23603 A (AKERMAN BEVERLY R ;UNIV MCGILL (CA); TREACY EILEEN P (CA)) 5 April 2001 (2001-04-05)

D2: BASARAB T ET AL: 'SEQUENCE VARIATIONS IN THE FLAVIN-CONTAINING MONO-OXYGENASE 3 GENE (FMO3) IN FISH ODOUR SYNDROME' BRITISH JOURNAL OF DERMATOLOGY, XX, XX, vol. 140, no. 1, January 1999 (1999-01), pages 164-167, XP001053043 ISSN: 0007-0963 cited in the application

D3: TREACY E P ET AL: 'MUTATIONS OF THE FLAVIN-CONTAINING MONOOXYGENASE GENE (FMO3) CAUSE TRIMETHYLAMINURIA, A DEFECT IN DETOXICATION' HUMAN MOLECULAR GENETICS, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 7, no. 5, May 1998 (1998-05),

pages 839-845, XP001053063 ISSN: 0964-6906 cited in the application

D4: DOLPHIN COLIN T ET AL: 'Structural organization of the human flavin-containing monooxygenase 3 gene (FMO3), the favored candidate for fish-odor syndrome, determined directly from genomic DNA.' GENOMICS, vol. 46, no. 2, 1 December 1997 (1997-12-01), pages 260-267, XP002204053 ISSN: 0888-7543 cited in the application

1. Novelty (Art. 33(2) PCT)

1.1 D1 describes human FMO3 gene mutations and polymorphisms and the detection of an altered metabolism of flavin-containing monooxygenase (FMO3) enzyme substrate or its isoform in a patient.

1.2 D2 describe FMO3 mutations in an individual with this disorder using polymerase chain reaction of genomic DNA, heteroduplex analysis and direct sequencing of heteroduplex band shifts. D2 identified a heterozygous missense Pro153-Leu153 mutation in exon 4. Leu153 has been reported previously as a homozygous mutation in two unrelated siblings with trimethylaminuria and has been shown to result in total loss of FMO3 enzyme activity.

1.3 D3 describes mutations in the human flavin containing monooxygenase isoform 3 gene(FMO3) which impair IV-oxygenation of xenobiotics and are responsible for the trimethylaminuria phenotype. Three disease-causing mutations in nine Australian-born probands have been identified which share a particular polymorphic haplotype.

1.4 D4 describes the structural organization of the human flavin-containing monooxygenase 3 gene (FMO3), the favoured candidate for fish-odour syndrome, determined directly from genomic DNA. Trimethylaminuria (fish-odour syndrome) is associated with defective hepatic N-oxidation of dietary-derived trimethylamine catalysed by flavin-containing monooxygenase (FMO). As FMO3 encodes the major form of FMO expressed in adult human liver, it represents the best candidate gene for the disorder. The structural organization of FMO3 was determined by sequencing the products of exon-to-exon and vectorette PCR, the latter through the use of vectorette libraries constructed directly from genomic DNA

1.4.1 In the light of D1-D4, the subject-matter of claim 1-14 is novel under Art. 33(2) PCT.

2. Inventive Step (Art. 33(3) PCT)

2.1 D1 describes human FMO3 gene mutations and polymorphisms and the detection of an altered metabolism of flavin-containing monooxygenase (FMO3) enzyme substrate or its isoform in a patient, detecting susceptibility of patient to FMO3 enzyme substrate or detecting predisposition of patient to hypertension or disorder associated with exposure to heteroatom-containing chemical compound, its intermediate or metabolite.

D1, regarded as the closest state of the art, differs from the subject-matter that it lacks the essential technical feature of the bovine FMO3 gene respectively mutated variants thereof and the detection of a polymorphism which is responsible for the fishy off-flavour in bulk milk. In the light of the prior art, the problem of underlying application is the provision of a further FMO3 gene from a non-human animal. The solution as provided by the applicant are the flavin-containing monooxygenase form 3 (FMO3) from bovine, a set of primer for amplifying said nucleic acid sequence, and the detection of a mutation of the FMO3 gene in an animal which is responsible for the fishy off-flavour in milk.

The combination of the technical features of the independent claim 1 is neither known from, nor rendered obvious by, the available prior art.

2.1.1 In the light of D1 the subject-matter of claims 1-14 does comprise an inventive step under Art. 33(3) PCT.

VII. Certain defects

1. The claims as a whole are not clear and concise and thus do not fulfil the requirements of Art. 6 PCT. The terms (i) **'under stringent condition'** in claim 10 and 13 are not specified.

2. The present application does not fulfil the requirements of Art. 6 PCT. The present application does not disclose a single working example of the isolated nucleic acid sequence comprising up to 500 kb of a 3' and/or 5' adjacent genomic DNA sequence, or the complement thereof, as clamed in claim 7.

AMENDED CLAIMS

1. A polypeptide which is a flavin-containing monooxygenase 3 (FMO3), wherein said FMO3 is a polypeptide comprising at least a sequence having at least 85% identity with the polypeptide sequence SEQ ID NO: 15.

2. A polypeptide according to claim 1, which polypeptide is a functionally altered mutant of flavin-containing monooxygenase 3 (FMO3) leading to a buildup of trimethylamine in an animal.

3. A polypeptide according to claim 2 resulting from a deletion, insertion, non-sense, or mis-sense mutation in a FMO3 gene.

4. A polypeptide according to claim 3 which is the R238X variant of the bovine FMO3.

5. An isolated nucleic acid sequence encoding a polypeptide according to any of claims 1-4, or the complement thereof.

6. An isolated nucleic acid sequence according to claim 5 which is the nucleic acid sequence shown in SEQ ID NO: 14.

7. An isolated nucleic acid sequence comprising at least a portion of a nucleic acid sequence encoding a polypeptide of any of claims 1-4, and up to 500 kb of a 3' and/or a 5' adjacent genomic DNA sequence, or the complement thereof.

8. A nucleic acid fragment selected from the group consisting of;
- a specific fragment of a nucleic acid sequence encoding a polypeptide according to any of claims 1-4,

- a specific fragment of a nucleic acid sequence according to claim 7,

- SEQ ID NO: 9,
- SEQ ID NO: 10,
- SEQ ID NO: 11,
- SEQ ID NO: 12,
- SEQ ID NO: 16,
- SEQ ID NO: 17, and
- SEQ ID NO: 18.

9. A set of primers for amplifying a nucleic acid sequence according to any of claims 5-7, comprising at least one primer selected from the group consisting of the nucleic acid fragments according to claim 8.

10. A method for detecting a mutation in the *FMO3* gene of an animal, with the exception of humans, where the mutation will cause an alteration in the metabolism of trimethylamine leading to a fish off-flavour in a food product of the animal or its offspring, wherein the method comprises:

- obtaining a sample of genomic DNA from the animal,
- amplifying a segment of said DNA spanning a polymorphic marker by PCR using a set of primers according to claim 9, a set of primers which specifically hybridise under stringent conditions with a nucleic acid sequence encoding a polypeptide according to any of claims 1-4, or a set of primers which specifically hybridise under stringent conditions with a nucleic acid sequence according to claim 7, and
- detecting in said amplified DNA the presence of an allele of a polymorphic marker associated with said mutation in the *FMO3* gene.

11. A method for detecting a nucleic acid sequence comprising a mutation in the *FMO3* gene of an animal, with the exception of humans, where the mutation will cause an alteration in the metabolism of trimethylamine leading to a fish off-flavour in a food product of the animal or its offspring, wherein the method comprises:

- obtaining a nucleic acid sample from the animal;
- determining the presence in said nucleic acid sample of a nucleic acid sequence encoding a mutated FMO3 polypeptide.

12. A method for detecting a nucleic acid sequence according to claim 11, wherein said nucleic acid sequence is detected by

- contacting said nucleic acid sample with a nucleic acid probe spanning said mutation under conditions of specific hybridisation between said probe and the mutant sequence to be detected; and
- detecting the hybridisation complex.

13. A method according to claim 11 or 12 wherein the presence of the nucleic acid sequence encoding said mutant polypeptide is determined by contacting the nucleic acid sample with a nucleic acid fragment according to claim 8, a nucleic acid fragment which specifically hybridises under stringent conditions with a nucleic acid sequence encoding a polypeptide according to any of claims 1-4, or a nucleic acid fragment which specifically hybridises under stringent conditions with a nucleic acid sequence according to claim 7.

14. A method according to claim 11 or 12 which further comprises PCR amplification from the nucleic acid sample, of a sequence comprising at least the portion of the *FMO3* sequence wherein the mutation is to be detected.